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ORIGINAL ARTICLE

Benzylidene/2-aminobenzylidene hydrazides: Synthesis, characterization and *in vitro* antimicrobial evaluation

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Abstract In this study a series of new mannich bases were synthesized and characterized by elemental and spectral (IR, ^{1}H NMR, ^{13}C NMR) studies. All the synthesized compounds were evaluated for their antimicrobial activity by broth dilution method against two Gram negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) and fungal strain (*Candida albicans* and *Aspergillus niger*). Preliminary pharmacological evaluation revealed that the compounds **βf, 3i, 3j, and 3k**) showed good activity against these strains. The result demonstrates the potential and importance of developing new mannich bases which would be effective against resistant bacterial and fungal strain.

1. Introduction

The incidence of microbial infection has increased on alarming levels over the world as a result of antimicrobial resistance in past 25 years. The rapid development of resistance to existing antibacterial and antifungal drugs posses a major threat to public health and it creates a serious challenge to the scientific community. In addition, the treatment of infectious diseases is more complicated in immuno-suppressed patients, such as those infected with the HIV, undergoing anticancer therapy and organ transplants. Consequently, there is a vital need for the development of new antimicrobial agents having potent activity against the resistant microorganisms (Koca et al., 2005; Bonde and Gaikwad, 2004; Yu and Huiyuan, 2002; Ram, 1988). Hydrazones have been reported to posses, antimicrobial (Rollas et al., 2002), antitubercular (Imramovsky et al., 2007; Janin, 2007), antileprotic (Buuhoi et al., 1956), anticonvulsant (Dimmock

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et al., 2000), analgesic (Lima et al., 2000), anti-inflammatory (Salgin-Goksen et al., 2007; Kalsi et al., 1990), antiplatelet (Silva et al., 2004), anticancer (Savini et al., 2004; Bijev, 2006) and antiviral (Abdel-Aal et al., 2006) activity.

Inspired by the above facts and in continuation of our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds (Deep et al., 2010a, b; Madhukar et al., 2009; Kumar et al., 2010), we hereby report the synthesis the novel derivatives of isoniazid and evaluated them for antimicrobial activity.

2. Materials and methods

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates kiesel gel 0.25 mm, 60G F 254, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by iodine vapors/ultraviolet light as visualizing agent. The IR spectra (υ, cm^{-1}) were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^1H NMR spectra (δ, ppm) were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C NMR spectra were recorded on in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Loba Chemie, Fluka and Aldrich.

3. Chemistry

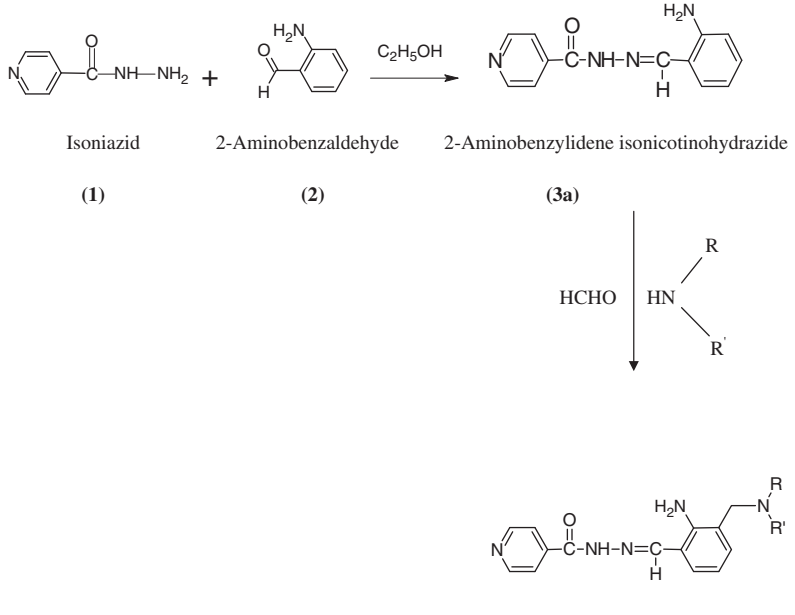
The synthesis of target compounds was carried outline in synthetic scheme (Scheme 1). Compounds **3a–3k** was readily prepared in good yields and purity. Equimolar quantity of 2-aminobenzaldehyde (**2**) and isoniazid (**1**) in 15 ml of absolute ethanol was refluxed for 7 h to form acid hydrazone. The completion of reaction was confirmed by thin layer chromatography (TLC). Then 2-aminobenzylidene isonicotinohydrazide (**3a**) along with formaldehyde and substituted secondary amines were refluxed for 34–42 h in presence of 50 ml of super dry ethanol (method of preparation of dry ethanol: take 1 l of ethanol and add 25 g of magnesium metals. Reflux until the metal is consumed (add a few drops of chloroform if it does not start to get cloudy). It will take a good 24 h to convert the metal to magnesium ethoxide. Then just distill the ethanol off. It will be very dry) and the pH was adjusted to 4 with hydrochloric acid. The types of substituted secondary amines are specified in Table 1. The synthesized novel mannich bases were characterized on the basis of the spectral and analytical studies.

3.1. Synthesis of 2-aminobenzylidene isonicotinohydrazide

A mixture of 2-aminobenzaldehyde (1.21 g, 0.01 mol) and isoniazid (1.37 g, 0.01 mol) in 15 ml of super dry ethanol was refluxed for 7 h. The completion of reaction was confirmed by TLC. The reaction mixture was then poured in ice cold water and the precipitate obtained was filtered and dried in oven at low temperature. The product was recrystallised from absolute ethanol.

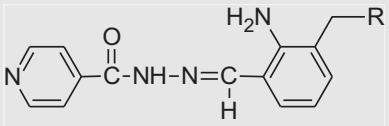
3.2. N-(2-Aminobenzylidine)isonicotinohydrazide (*3a*)

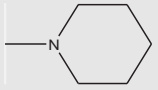
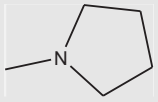
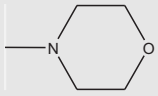
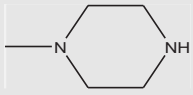
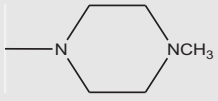
Yield 58%; m.p. 205–208 °C; IR (KBr; cm^{-1}): 3465, 3275, 3181, 2985, 2857, 2849, 1674, 1648, 1557, 1085. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 11.95 (s, 1H, –NH–N═), 8.65 (d, 2H, pyridine, J = 4.1 Hz), 8.37 (s, 1H, –N═C–H), 7.94 (d, 2H, pyridine, J = 3.7 Hz), 7.69 (d, 2H, benzylidene, J = 8.2 Hz), 7.37 (d, 2H, benzylidene, J = 7.8 Hz), 5.42 (s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.54, 161.18, 149.83,



Scheme 1 Synthetic pathway for the formation of the title compounds.

Table 1 Physical data of synthesized mannich bases.



Compounds	R	Molecular formulae	Yield (%)	Mp (°C)
3b	$-N(CH_3)_2$	$C_{16}H_{29}N_5O$	48	222–225
3c	$-N(C_2H_5)_2$	$C_{18}H_{23}N_5O$	52	215–218
3d	$-N(C_3H_7)_2$	$C_{20}H_{27}N_5O$	45	210–213
3e	$-N(C_4H_9)_2$	$C_{22}H_{31}N_5O$	48	208–211
3f	$-N(C_6H_5)_2$	$C_{26}H_{23}N_5O$	53	196–199
3g		$C_{19}H_{23}N_5O$	57	188–190
3h		$C_{18}H_{21}N_5O$	55	198–201
3i		$C_{18}H_{21}N_5O_2$	42	219–222
3j		$C_{18}H_{22}N_6O$	47	206–209
3k		$C_{19}H_{24}N_6O$	46	211–214

143.37, 139.85, 132.68, 130.15, 122.85, 121.23, 118.59, 115.84. Anal. Calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.78; H, 4.62; N, 17.34.

*3.3. Synthesis of substituted mannich bases (**3b**–**3k**)*

The 2-aminobenzylidene isonicotinohydrazide (576 mg, 0.0024 mol) along with (0.1 ml, 0.0036 mol) of formaldehyde and (0.0024 mol) of substituted secondary amines was placed in 100 ml round bottomed flask to which 50 ml of super dry ethanol was added and the pH was adjusted to 4 with hydrochloric acid and refluxed for 28–33 h. The completion of reaction was confirmed by TLC. The reaction mixture was then poured into beaker and concentrated on water bath. The reaction mixture was allowed to cool at room temperature and then in which diethyl ether was added. The reaction mixture was kept for 3–5 h in refrigerator and filtered and washed with *n*-hexane. The products were recrystallised from absolute ethanol (Sriram et al., 2005).

*3.4. N-3-((Dimethyamino) methyl)-2-aminobenzylidene)-isonicotinohydrazide (**3b**)*

IR (KBr; cm^{-1}): 3348, 3265, 3185, 2965, 2863, 2842, 1674, 1645, 1566, 1082. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 11.95 (s, 1H, –NH–N═), 8.69 (d, 2H, pyridine, J = 4.2 Hz), 8.46 (s, 1H, –N═C–H), 7.89 (d, 2H, pyridine, J = 3.8 Hz), 7.72 (d, 1H, benzylidene, J = 3.2 Hz), 7.24 (t, 1H, benzylidene), 4.25 (s, 2H, NH_2, D_2O exchangeable), 3.59 (s, 2H, Ar–CH_2–N), 2.18 (s, 6H, N–2CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.59, 149.62, 148.57, 143.29, 139.87, 132.54, 128.52, 124.57, 122.49, 118.72, 114.87, 56.64, 46.89. Anal. Calcd. for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.68; H, 6.47; N, 23.47.

*3.5. N-3-((Diethyamino) methyl)-2-aminobenzylidene)-isonicotinohydrazide (**3c**)*

IR (KBr; cm^{-1}): 3445, 3268, 3175, 2974, 2864, 2843, 1676, 1648, 1565, 1074. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 11.92 (s, 1H, –NH–N═), 8.54 (d, 2H, pyridine, J = 4.2 Hz), 8.52 (s, 1H, –N═C–H), 7.85 (d, 2H, pyridine, J = 3.9 Hz), 7.65 (d, 2H, benzylidene, J = 3.2 Hz), 7.12 (t, 1H, benzylidene), 4.11 (s, 2H, NH_2, D_2O exchangeable), 3.55 (s, 2H, Ar–CH_2–N), 2.25 (m, 4H, N–2CH_2), 1.18 (m, 6H, 2CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.29, 149.85, 148.24, 143.13, 139.81, 132.19, 128.72, 124.59, 122.57, 118.53, 114.71, 52.17, 49.28, 14.75. Anal. Calcd. for $C_{18}H_{23}N_5O$: C, 66.44; H, 7.12; N, 21.52. Found: C, 66.28; H, 7.25; N, 21.55.

*3.6. N-3-((Dipropylamino)methyl)-2-aminobenzylidene)-isonicotinohydrazide (**3d**)*

IR (KBr; cm^{-1}): 3452, 3255, 3188, 2982, 2863, 2844, 1677, 1647, 1561, 1078. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 11.88 (s, 1H, –NH–N═), 8.59 (d, 2H, pyridine, J = 4.1 Hz), 8.47 (s, 1H, –N═C–H), 7.69 (d, 2H, pyridine, J = 3.8 Hz), 7.45 (d, 2H, ben-

zylidene, J = 3.1 Hz), 7.38 (t, 1H, benzylidene), 4.35 (s, 2H, NH_2, D_2O exchangeable), 3.81 (s, 2H, Ar–CH_2–N), 2.35 (t, 4H, N–2CH_2), 1.56 (m, 4H, 2CH_2), 1.12 (m, 6H, 2CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.59, 149.74, 148.12, 143.18, 139.74, 132.34, 128.59, 124.52, 122.65, 118.64, 114.79, 57.24, 52.18, 22.55, 13.72. Anal. Calcd. for $C_{20}H_{27}N_5O$: C, 67.96; H, 7.70; N, 19.81. Found: C, 67.95; H, 7.64; N, 19.88.

*3.7. N-3-((Dibutylamino)methyl-2-aminobenzylidene)-isonicotinohydrazide (**3e**)*

IR (KBr; cm^{-1}): 3452, 3292, 3178, 2971, 2864, 2845, 1668, 1644, 1558, 1072. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.85 (s, 1H, –NH–N═), 8.55 (d, 2H, pyridine, J = 4.1 Hz), 8.49 (s, 1H, –N═C–H), 7.85 (d, 2H, pyridine, J = 3.7 Hz), 7.78 (d, 2H, benzylidene, J = 3.2 Hz), 7.42 (t, 1H, benzylidene), 4.18 (s, 2H, NH_2, D_2O exchangeable), 3.64 (s, 2H, Ar–CH_2–N), 2.32 (t, 4H, N–2CH_2), 1.35 (m, 8H, 4CH_2), 1.15 (t, 6H, 2CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.54, 149.45, 143.35, 139.78, 132.64, 128.55, 124.59, 122.68, 118.65, 114.88, 55.72, 52.18, 32.67, 21.15, 15.71. Anal. Calcd. for $C_{22}H_{31}N_5O$: C, 69.28; H, 8.12; N, 18.40. Found: C, 69.25; H, 8.19; N, 18.36.

*3.8. N-(3((Diphenylamino)-methyl)-2-aminobenzylidene)-isonicotinohydrazide (**3f**)*

IR (KBr; cm^{-1}): 3446, 3269, 3185, 2967, 2859, 2842, 1674, 1645, 1549, 1074. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.95 (s, 1H, –NH–N═), 8.69 (d, 2H, pyridine, J = 4.2 Hz), 8.52 (s, 1H, –N═C–H), 7.85 (d, 2H, pyridine, J = 3.9 Hz), 7.89–6.92 (m, 13H, benzylidene), 4.25 (s, 2H, NH_2, D_2O exchangeable), 3.78 (s, 2H, Ar–CH_2–N); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.18, 149.87, 148.22, 143.58, 139.65, 132.59, 128.59, 124.55, 122.78, 119.18, 117.53, 114.29, 47.81. Anal. Calcd. for $C_{26}H_{23}N_5O$: C, 74.09; H, 5.50; N, 16.62. Found: C, 74.13; H, 5.55; N, 16.53.

*3.9. N-(2-Amino-3-((piperidine-1-yl)methyl)benzylidene)-isonicotinohydrazide (**3g**)*

IR (KBr; cm^{-1}): 3458, 3294, 3175, 2984, 2862, 2844, 1678, 1648, 1555, 1077. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.78 (s, 1H, –NH–N═), 8.58 (d, 2H, pyridine, J = 4.1 Hz), 8.45 (s, 1H, –N═C–H), 7.88 (d, 2H, pyridine, J = 3.8 Hz), 7.75 (d, 2H, benzylidene, J = 3.1 Hz), 7.45 (t, 1H, benzylidene), 4.28 (s, 2H, NH_2, D_2O exchangeable), 3.51 (s, 2H, Ar–CH_2–N), 2.24 (t, 4H, N–2CH_2, piperidine), 1.82 (m, 6H, 3CH_2, piperidine); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.44, 149.65, 148.27, 143.38, 139.63, 132.54, 128.41, 124.58, 121.72, 118.64, 114.61, 55.64, 52.37, 27.18. Anal. Calcd. for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; N, 20.67. Found: C, 67.68; H, 6.85; N, 20.64.

*3.10. N-(2-Amino-3-((pyrrolidin-1-yl)methyl)benzylidene)-isonicotinohydrazide (**3h**)*

IR (KBr; cm^{-1}): 3462, 3281, 3178, 2983, 2865, 2838, 1675, 1642, 1552, 1073. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.83 (s, 1H, –NH–N═), 8.68 (d, 2H, pyridine, J = 4.1 Hz), 8.43 (s, 1H, –N═C–H), 7.85 (d, 2H, pyridine, J = 3.9 Hz), 7.82 (d, 2H, benzylidene, J = 3.2 Hz), 7.32 (t, 1H, benzylidene), 4.15 (s, 2H, NH_2, D_2O exchangeable), 3.55 (s, 2H, Ar–CH_2–N), 2.32 (m, 4H, N–2CH_2, pyrrolidine), 1.58 (m, 4H, 2CH_2, pyrrolidine); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.48, 149.66, 148.28, 143.29, 139.62, 132.55, 128.54, 124.51, 122.75, 118.69, 114.64, 58.84, 52.18, 25.18. Anal. Calcd. for $C_{18}H_{21}N_5O$: C, 66.85; H, 6.55; N, 21.66. Found: C, 66.73; H, 6.72; N, 21.61.

*3.11. N-(2-Amino-3-((morpholinomethyl)benzylidene)-isonicotinohydrazide (**3i**)*

IR (KBr; cm^{-1}): 3465, 3278, 3172, 2985, 2861, 2847, 1674, 1644, 1551, 1085. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.89 (s, 1H, –NH–N═), 8.65 (d, 2H, pyridine, J = 4.2 Hz), 8.37 (s, 1H, –N═C–H), 7.85 (d, 2H, pyridine, J = 3.8 Hz), 7.81 (d, 2H, benzylidene, J = 3.1 Hz), 7.35 (t, 1H, benzylidene), 4.18 (s, 2H, NH_2, D_2O exchangeable), 3.58 (s, 2H, Ar–CH_2–N), 3.42 (m, 4H, O–2CH_2, morpholine), 2.35 (t, 4H, N–2CH_2, morpholine); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.65, 149.84, 148.15, 143.35, 139.68, 132.47, 128.43, 122.64, 118.74, 114.72, 68.52, 55.71, 52.18. Anal. Calcd. for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64. Found: C, 63.84; H, 6.13; N, 20.61.

*3.12. N-(2-Amino-3-((piperazin-1-yl) methyl)benzylidene)-isonicotinohydrazide (**3j**)*

IR (KBr; cm^{-1}): 3462, 3274, 3171, 2988, 2862, 2843, 1672, 1641, 1554, 1082. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.88 (s, 1H, –NH–N═), 8.68 (d, 2H, pyridine, J = 4.1 Hz), 8.35 (s, 1H, –N═C–H), 7.82 (d, 2H, pyridine, J = 3.7 Hz), 7.84 (d, 2H, benzylidene, J = 3.2 Hz), 7.29 (t, 1H, benzylidene), 4.15 (s, 2H, NH_2, D_2O exchangeable), 3.55 (s, 2H, Ar–CH_2–N), 2.65–2.48 (m, 8H, 4CH_2, piperazine); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.51, 149.86, 148.27, 143.49, 139.67, 132.58, 128.38, 122.49, 118.63, 114.78, 55.81, 52.77, 47.18. Anal. Calcd. for $C_{18}H_{22}N_6O$: C, 63.89; H, 6.55; N, 24.83. Found: C, 63.72; H, 6.69; N, 24.86.

*3.13. N-(2-Amino-3-((4-methylpiperazin-1-yl)methyl)-benzylidene)isonicotinohydrazide (**3k**)*

IR (KBr; cm^{-1}): 3462, 3274, 3175, 2982, 2863, 2842, 1675, 1643, 1555, 1088. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.93 (s, 1H, –NH–N═), 8.67 (d, 2H, pyridine, J = 4.2 Hz), 8.19 (s, 1H, –N═C–H), 7.83 (d, 2H, pyridine, J = 3.7 Hz), 7.66 (d, 2H, benzylidene, J = 3.2 Hz), 7.18 (t, 1H, benzylidene), 4.32 (s, 2H, NH_2, D_2O exchangeable), 3.72 (s, 2H, Ar–CH_2–N), 2.45 (m, 8H, 4CH_2, piperazine), 2.13 (s, 3H, CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 163.39, 149.87, 148.11, 143.37, 139.61, 132.39, 128.45, 122.81, 118.24, 114.67, 58.22, 53.14, 52.19, 44.39. Anal. Calcd. for $C_{19}H_{24}N_6O$: C, 64.75; H, 6.86; N, 23.85. Found: C, 64.67; H, 6.81; N, 23.98.

4. Antimicrobial evaluation

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against Gram positive bacteria: *Staphylococcus aureus* (MTCC 121), *Bacillus subtilis* (MTCC 96), Gram negative *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa*

Table 2 Antimicrobial screening results of the tested compounds.

Compound	Minimum inhibitory concentration (μg ml^{-1})					
	Gram positive bacteria		Gram negative bacteria		Fungal strain	
	B. subtilis (MTCC 96)	*S. aureus* (MTCC 121)	*P. aeruginosa* (MTCC 2453)	*E. coli* (MTCC 40)	*C. albicans* (MTCC 8184)	*A. niger* (MTCC 8184)
3a	12.5	25	12.5	6.25	12.5	12.5
3b	12.5	25	3.12	6.25	25	50
3c	6.25	25	50	>100	12.5	12.5
3d	3.12	12.5	12.5	25	50	12.5
3e	25	50	12.5	6.25	3.12	12.5
3f	3.12	1.56	3.12	1.56	3.12	3.12
3g	25	50	25	12.5	12.5	50
3h	6.25	25	12.5	6.25	12.5	25
3i	1.56	3.12	3.12	3.12	1.56	3.12
3j	3.12	3.12	1.56	1.56	3.12	3.12
3k	3.12	6.25	1.56	1.56	12.5	1.56
Amoxicillin	0.15	0.15	0.25	0.15	–	–
Nystatin	–	–	–	–	0.25	0.78

(MTCC 2453) and fungal strain: *Candida albicans* (MTCC 8184) and *Aspergillus niger* (MTCC 8189). Antimicrobial activity was assessed by serial twofold dilution technique. Amoxicillin was used as a standard drug for antibacterial activity while Nystatin was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 μg ml^{-1}. Twofold dilutions of test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) (Pharmacopoeia, 1996). The stock solution was serially diluted to give concentrations of 100–0.78 μg ml^{-1} in nutrient broth. The inoculum size was approximately 10^6 colony forming units (CFU/ml). The tubes were incubated at 37 ± 1 °C for 24 h (bacteria) and 25 °C for 7 days (*A. niger*). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for antimicrobial was given in Table 2.

5. Results and discussion

In this study novel mannich bases have been synthesized and evaluated them for antimicrobial activity. In general, IR spectra of all compound **3a–3k** showed absorption band at around 3465–3165, 3240–3255, 2988–2965, 2865–2838, 1678–1668, 1648–1641, 1566–1549, and 1088–1072 cm^{-1} regions, conforming the presence of NH_2, NH, CH, CH_2, C═N, C═O, C═C, and C–N, respectively. The ^{1}H NMR spectra, the signals of the respective prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of most compounds showed the characteristic NH proton δ 11.95–11.78 ppm, 1H proton of –N═C–H at δ 8.52–8.25 ppm, 4H proton of pyridine were at around δ 8.89–7.65 ppm, characteristic protons of benzylidene at δ 7.89–6.94 ppm, 2H proton of NH_2 at δ 5.42–4.11 ppm and 2H proton of Ar–CH_2–N at δ 3.81–3.51 ppm, ^{13}C NMR spectra of most compounds have characteristic C═O signals appeared at around δ 163.18–163.65 ppm, pyridine δ 149.87–122.49 ppm, –N═C–H δ 143.58–143.18 ppm, benzylidene δ 161.18–114.29 ppm, Ar–CH_2–N δ 56.15–50.67 ppm. The elemental analysis, IR and ^{1}H NMR, ^{13}C NMR spectral data of synthesized compounds were found in agreement with the assigned molecular structure. Among the synthesized derivatives, compounds (**3f, 3i, 3j,** and **3k**) were the most active derivatives against these strains as compared to the standard drugs. So, it was concluded that the presence of diphenyl amine, morpholine, piperazine and N-methyl piperazine moiety besides pyridine ring was found to be essential for their high antibacterial and antifungal activity. It was also concluded from the results that antimicrobial activity increases with increase in chain length from dimethyl amine to dibutyl amine. So, the significant antimicrobial activity of compound may be due to the presence of diphenyl amine, morpholine, piperazine and N-methyl piperazine moiety in addition to hydrazide functional group.

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